Tissue-specific alterations in thyroid hormone homeostasis in combined Mct10 and Mct8 deficiency

Müller, Julia ; Mayerl, Steffen ; Visser, Theo J ; Darras, Veerle M ; Boelen, Anita ; Frappart, Lucien ; Mariotta, Luca ; Verrey, Francois ; Heuer, Heike

Abstract: The monocarboxylate transporter Mct10 (Slc16a10; T-type amino acid transporter) facilitates the cellular transport of thyroid hormone (TH) and shows an overlapping expression with the well-established TH transporter Mct8. Because Mct8 deficiency is associated with distinct tissue-specific alterations in TH transport and metabolism, we speculated that Mct10 inactivation may compromise the tissue-specific TH homeostasis as well. However, analysis of Mct10 knockout (ko) mice revealed normal serum TH levels and tissue TH content in contrast to Mct8 ko mice that are characterized by high serum T3, low serum T4, decreased brain TH content, and increased tissue TH concentrations in the liver, kidneys, and thyroid gland. Surprisingly, mice deficient in both TH transporters (Mct10/Mct8 double knockout [dko] mice) showed normal serum T4 levels in the presence of elevated serum T3, indicating that the additional inactivation of Mct10 partially rescues the phenotype of Mct8 ko mice. As a consequence of the normal serum T4, brain T4 content and hypothalamic TRH expression were found to be normalized in the Mct10/Mct8 dko mice. In contrast, the hyperthyroid situation in liver, kidneys, and thyroid gland of Mct8 ko mice was even more severe in Mct10/Mct8 dko animals, suggesting that in these organs, both transporters contribute to the TH efflux. In summary, our data indicate that Mct10 indeed participates in tissue-specific TH transport and also contributes to the generation of the unusual serum TH profile characteristic for Mct8 deficiency.

DOI: https://doi.org/10.1210/en.2013-1800

Tissue-Specific Alterations in Thyroid Hormone Homeostasis in Combined Mct10 and Mct8 Deficiency

Julia Müller, Steffen Mayerl, Theo J. Visser, Veerle M. Darras, Anita Boelen, Lucien Frappart, Luca Mariotta, Francois Verrey, and Heike Heuer

Leibniz Institute for Age Research/Fritz Lipmann Institute (J.M., S.M., L.F., H.H.), Jena, Germany; Department of Internal Medicine (T.J.V.), Erasmus Medical Center, Rotterdam, The Netherlands; Laboratory of Comparative Endocrinology (V.M.D.), Biology Department, Katholieke Universiteit Leuven, Leuven, Belgium; Department of Endocrinology and Metabolism (A.B.), Academic Medical Center, Amsterdam, The Netherlands; Institute of Physiology and Zürich Center for Integrative Human Physiology (L.M., F.V.), University of Zürich, Zürich, Switzerland; and Leibniz Institute for Environmental Medicine (H.H.), Düsseldorf, Germany

The monocarboxylate transporter Mct10 (*Slc16a10*; T-type amino acid transporter) facilitates the cellular transport of thyroid hormone (TH) and shows an overlapping expression with the well-established TH transporter Mct8. Because Mct8 deficiency is associated with distinct tissue-specific alterations in TH transport and metabolism, we speculated that Mct10 inactivation may compromise the tissue-specific TH homeostasis as well. However, analysis of Mct10 knockout (ko) mice revealed normal serum TH levels and tissue TH content in contrast to Mct8 ko mice that are characterized by high serum T_3, low serum T_4, decreased brain TH content, and increased tissue TH concentrations in the liver, kidneys, and thyroid gland. Surprisingly, mice deficient in both TH transporters (Mct10/Mct8 double knockout [dko] mice) showed normal serum T_4 levels in the presence of elevated serum T_3, indicating that the additional inactivation of Mct10 partially rescues the phenotype of Mct8 ko mice. As a consequence of the normal serum T_4, brain T_4 content and hypothalamic TRH expression were found to be normalized in the Mct10/Mct8 dko mice. In contrast, the hyperthyroid situation in liver, kidneys, and thyroid gland of Mct8 ko mice was even more severe in Mct10/Mct8 dko animals, suggesting that in these organs, both transporters contribute to the TH efflux. In summary, our data indicate that Mct10 indeed participates in tissue-specific TH transport and also contributes to the generation of the unusual serum TH profile characteristic for Mct8 deficiency. **(*Endocrinology* 155: 315–325, 2014)**

Thyroid hormone (TH) action and metabolism require the transport of TH across cell membranes because TH receptors as well as the TH metabolizing deiodinases exert their action intracellularly. Until now, several proteins have been identified that can facilitate TH passage in and out of cells (1). Among them, the monocarboxylate transporter 8 (MCT8) has received greatest attention because MCT8 only accepts TH as substrate, thereby exhibiting the most restricted substrate specificity (2). Moreover, inactivating mutations in the X-linked *MCT8* gene are associated with a severe form of psychomotor retardation, also known as Allan-Herndon-Dudley syndrome (3–5). In addition to pronounced neurologic symptoms and muscle hypotonia, patients with inactive MCT8 exhibit very unusual circulating TH values with highly elevated T_3 in the presence of low to normal T_4 (6). These endocrine abnormalities are fully replicated in Mct8 knockout (ko) animals (7, 8). In contrast, Mct8 ko mice do not show any overt neurologic abnormalities although the uptake of T_3 into the central nervous system (CNS) is strongly impaired in the absence of Mct8 (8–10). However, T_4 can still enter the CNS in these animals by taking advantage of the T_4-

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in U.S.A.

Received August 25, 2013. Accepted November 1, 2013.
First Published Online November 18, 2013

Abbreviations: BBB, blood-brain barrier; CNS, central nervous system; D1 and D2, type 1 and 2 deiodinases; dko, double knockout; ISH, in situ hybridization; ko, knockout; MCT, monocarboxylate transporter; P21, 21 days old; PBF, Pttg1-binding factor; PTC, papillary thyroid carcinoma; qPCR, quantitative real-time PCR; TAT, T-type amino acid transporter; TH, thyroid hormone; Wt, wild type.

specific transporter Oatp1c1 that in rodents is highly expressed in the choroid plexus and in blood-brain-barrier (BBB) endothelial cells (11, 12) but absent in BBB endothelial cells of primates (13) and only weakly expressed in the human CNS (14). That Mct8 and Oatp1c1 indeed act together in providing TH access to the brain could only recently be demonstrated by the analysis of Mct8/Oatp1c1 double knockout (dko) mice. These animals show an abolished uptake of both T_3 and T_4 into the brain (15), indicating that the lack of one transporter might be compensated by the presence of another carrier present in the same cell.

In addition to its crucial role in the CNS, human and mouse Mct8 is widely expressed in many peripheral tissues such as liver, kidney, and thyroid where it may also contribute substantially to tissue-specific TH transport. Analysis of the tissue TH content revealed strongly increased TH concentrations in the kidneys and thyroid gland of Mct8 ko mice, suggesting that Mct8 localized at the basolateral membrane preferentially acts as a TH efflux system (16–18). Moreover, highly elevated type 1 deiodinase (D1) activities in liver and kidneys indicate a thyrotoxic state of these organs in the absence of Mct8 (7, 8, 17). Consequently, in addition to Mct8, at least one other TH transporter must be present that eventually facilitates the access of TH into these cells.

As a putative candidate we considered the monocarboxylate transporter 10 (Mct10, also known as T-type amino acid transporter TAT1) as another transporter that may significantly contribute to TH transport in vivo. Among all members of the monocarboxylate transporter family Mct8 and Mct10 are most closely related (19). Moreover, Mct10 was demonstrated not only to facilitate the transport of Phe, Tyr, Trp, and Dopa (19–21) but also to accept iodothyronines as substrates (22). Expression studies in mice revealed the localization of Mct10 protein at the basolateral membrane of small intestine epithelial cells, of renal proximal tubule cells, and in perivenous hepatocytes (19, 23), indicating an overlapping expression pattern of Mct10 and Mct8 in kidneys and liver (18, 19).

The physiological relevance of Mct10 for amino acid homeostasis was recently addressed in studies of Mct10 ko animals (24). Like Mct8 ko mice, Mct10-deficient animals appear normal with respect to growth and fertility, and they also do not exhibit overt neurologic symptoms. Determination of aromatic amino acid concentrations, however, revealed increased levels in plasma, muscle, and kidneys whereas liver aromatic amino acid content remained unaltered. To which degree TH transport and tissue concentrations are affected by the absence of Mct10 has not yet been addressed.

Here, we report on our findings regarding TH transport, metabolism, and action in Mct10 ko mice as well as in animals lacking both transporters, Mct10 and Mct8 (Mct10/Mct8 dko mice). Whereas TH homeostasis was found to be rather unaffected by the absence of Mct10, Mct10/Mct8 dko animals exhibited distinct alterations in tissue TH content that were strikingly different from those changes found in Mct8 ko animals. Most intriguingly, low serum and brain T_4 concentrations, a hallmark of Mct8 ko mice, were normalized in Mct10/Mct8 dko animals, suggesting that the additional inactivation of Mct10 partially rescues the TH abnormalities characteristic for Mct8 deficiency.

Materials and Methods

Animals

Female Mct8 +/− mice were obtained from Deltagen and used to generate Mct8 ko mice as previously described (8). The Mct10 ko mouse was generated by Ingenium Pharmaceuticals AG. By *N*-ethyl-nitrosourea mutagenesis, a nonsense mutation was introduced into the Mct10 gene that leads to a premature stop codon at amino acid 88 (Y88X). Absence of Mct10 expression in these animals was previously confirmed by immunofluorescence and mRNA analysis (24). Genotyping was performed by PCR (35 cycles, 30 seconds at 95°C, 60 seconds at 55°C, and 30 seconds at 72°C) using the following primers (Frw: 5′-GGG ACC CTC GGA TGT CTC-3′, Rev: 5′-CAC CCT CCC AAC ACA AGA-3′). The PCR product was then subjected to enzymatic restriction using DdeI (2 hours at 37°C) in order to detect the point mutation in the Mct10 mutated allele after gel electrophoresis. Expected size of the products after digestion: 320 bp for the wild-type (Wt) allele and 170 bp for the mutant allele.

Mct10/Mct8 dko mice were produced by mating the respective single knockout mice and consecutively Mct10/Mct8 heterozygous animals. All mice used in these studies were males on a C57BL/6 background, and all animal studies were approved by the Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz (Thüringen, Bad Langensalza, Germany). Details concerning tissue collection are provided as supplemental data.

Serum TH levels were determined by in-house RIAs as reported elsewhere (25, 26). Serum alkaline phosphatase and cholesterol concentrations were determined by Laboklin. Tissue T_4 and T_3 content was analyzed after extraction as described previously (27). TSH serum levels were determined using a double-antibody precipitation RIA as published previously (28, 29) and described briefly in the supplemental data.

In situ hybridization (ISH)

The list of cDNA fragments used for the generation of ^{35}S-uridine 5-triphosphate (Hartmann Analytik) radiolabeled or Digoxigenin-UTP-labeled riboprobes by in vitro transcription is provided in Supplemental Table 1 published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org. ISH was carried out as published previously (30) and briefly described in the supplemental data. Experiments carried

out using the respective sense probes did not produce any hybridization signals (data not shown).

Quantitative real-time PCR (qPCR)

Total RNA from pituitary and thyroid was isolated using RNeasy Lipid Tissue Mini Kit (QIAGEN) and from liver and kidney by using Nucleospin RNA II Kit (Macherey-Nagel) according to the manufacturer's instructions and stored at −80 C. Synthesis of cDNA was performed using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche). To exclude the presence of genomic DNA, one sample without reverse transcriptase was included as well. qPCR was performed using the iQ SYBR Green Supermix and the Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories). At least 5 samples per genotype were subjected to the analysis. Cyclophilin was used as a housekeeping gene for normalization. A list of primers used for the generation of the respective PCR fragments is provided in Supplemental Table 2.

Thyroid histology

For morphologic analysis, thyroid glands were isolated and fixed in phosphate-buffered 4% formalin at room temperature overnight and rinsed in PBS and increasing ethanol concentrations. Thyroids were embedded in paraffin and cut on a microtome. In order to quantify the thyroid follicle areas, 5-μm thick paraffin sections from at least 3 thyroids per genotype and time point were counterstained with hematoxylin/eosin according to standard protocols. Using 3–4 sections of each thyroid gland, the area of all follicles was determined using the open source ImageJ software (NIH, Bethesda, Maryland).

For Ki-67 immunostaining, thyroid paraffin sections were deparaffinized and treated with 1% NaOH and 1% H_2O_2 to block the endogenous peroxidase activity. Then, sections were boiled for 20 minutes in citrate buffer (pH 6.0) to perform antigen retrieval. After blocking in 0.2% PBS-Tween 20 containing 10% normal goat serum at room temperature for 1 hour, sections were incubated with a primary antibody against Ki-67 (1:300; monoclonal rat; eBioscience) at 4°C overnight, and then washed in PBS and incubated for 1 hour with a biotinylated secondary antibody (Vector Laboratories). For signal detection, sections were treated 30 minutes with ABC Elite reagent (Vectastain) and subsequently stained with diaminobenzidine/peroxide (SurModics).

In vivo TH transport studies

Adult animals (3–5 animals per genotype and time point) were injected ip with 2.5×10^6 cpm [^{125}I]T_4 (PerkinElmer). After 1 hour, 3 hours, and 5 hours animals were anesthetized with isoflurane, blood samples were collected, and subsequently the mice were perfused with saline. After removal of the organs, the amount of radioactivity in the respective tissue was measured by an automatic γ-counter. Results were presented as the percentage of the injected dose per g tissue.

Statistical analysis

If not stated otherwise, the values represent mean ± SEM. Statistical significance between groups was determined by two-way ANOVA followed by a post hoc test (Bonferroni). $P < .05$ was considered to be statistically significant.

Results

For studying the functional significance of Mct10 in TH homeostasis, $Mct10^{-/-}/Mct8^{+/-}$ female mice were mated with $Mct10^{-/-}/Mct8^{-/y}$ male mice. Because Mct8 is localized on the X chromosome, male offspring are either $Mct10^{-/-}/Mct8^{+/y}$ (= Mct10 ko) or $Mct10^{-/-}/Mct8^{-/y}$ (= Mct10/Mct8 dko). In addition, male Wt and Mct8 ko mice were included in the analysis that were generated by mating heterozygous Mct8 females with Mct8 ko males. All breeding pairs produced offspring with the expected Mendelian ratio excluding prenatal lethality. Pups of all genotypes developed normally and did not show any signs of growth retardation. Animals of all genotypes and both sexes were fertile and phenotypically normal.

Analysis of serum TH profile and activity of the hypothalamus-pituitary system

Analysis of serum T_4 levels of 21-day-old (P21) and 2.5-months-old animals revealed no significant differences between Wt and Mct10 ko mice whereas a slight decrease in serum T_3 could be observed in Mct10 ko mice at P21 but not at the age of 2.5 months. In comparison, Mct8 ko mice exhibited the expected abnormalities with decreased serum T_4 and increased serum T_3 that even increased with age (Figure 1). Similarly elevated serum T_3 levels could also be observed in Mct10/Mct8 dko animals. Interestingly, Mct10/Mct8 dko showed only a mild reduction in serum T_4 at P21 and even similar levels as Wt and Mct10 ko mice at the age of 2.5 months. These data indicate that the additional inactivation of Mct10 partially rescues the TH abnormalities of Mct8-deficient mice.

How do the changes in TH serum profile affect the tissue-specific TH homeostasis? Determination of the TH content in forebrains isolated from 2.5-month-old animals revealed normal T_3 and T_4 values in Mct10 ko mice whereas Mct8 ko mice exhibited an approximately 50% reduction in both T_3 and T_4 concentrations (Figure 2A). Interestingly, TH forebrain content was only mildly decreased in Mct10/Mct8 dko mice, indicating again a partial rescue of the Mct8 phenotype in the double-mutant animals.

In order to assess the impact of the combined transporter deficiency on the activity of the hypothalamus-pituitary-thyroid axis, we analyzed TRH transcript levels in the paraventricular hypothalamic nucleus as well as TSH transcript levels in pituitaries by nonradioactive ISH. As shown in Figure 2B, signal intensities were not different between Wt and Mct10 ko mice, suggesting rather normal TRH and TSH expression levels in the absence of Mct10. In agreement with previous findings, Mct8 ko mice showed increased TRH and TSH transcript levels, indicating a hypothyroid situation in both hypothalamus and pituitary. In Mct10/Mct8 dko mice,

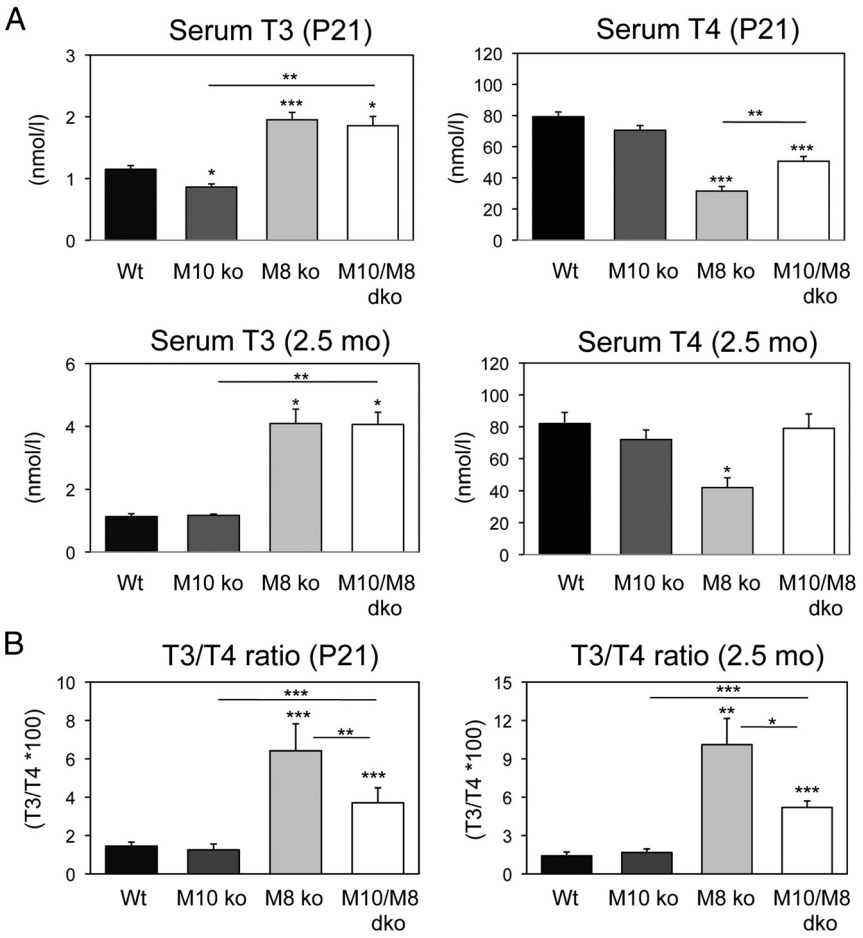

Figure 1. Abnormal serum TH profile of Mct8 ko mice is partially normalized in Mct10/Mct8-deficient animals A, Serum TH concentrations were determined at P21 (n = 7–10 per genotype) and at the age of 2.5 months (n = 5 per genotype). Whereas Mct10 (M10) ko animals showed rather normal serum TH levels, Mct10/Mct8 dko (M10/M8 dko) mice exhibited the same abnormally elevated serum T_3 levels as Mct8 (M8) ko animals. Unexpectedly, serum T_4 concentrations were significantly higher in the M10/M8 dko animals compared with Mct8 ko mice. Consequently, the T_3/T_4 ratio is less disturbed in the absence of Mct10 and Mct8 as illustrated in panel B. *, $P < .05$; **, $P < .005$; ***, $P < .001$.

TSH levels were found to be similarly elevated. However, hypothalamic TRH levels were not obviously up-regulated in these double-mutant animals.

Enhanced pituitary TSH expression in Mct8 ko and Mct10/Mct8 dko mice could be further confirmed by quantitative real-time PCR (qPCR) (Figure 2C). Transcript levels of D2 and GH were not altered in any animal group. Intriguingly, prolactin transcript levels were found to be highly elevated in Mct8 ko mice. This increase in pituitary prolactin may be a consequence of the elevated hypothalamic TRH levels because TRH acts also on lactotrophs, thereby stimulating prolactin synthesis (31, 32).

Impact of combined Mct10/Mct8 deficiency on thyroid gland activity

Next, we addressed the consequences of a combined Mct10/Mct8 deficiency on thyroid function and morphology. Both Mct10 and Mct8 are expressed in follicular epithelial cells as demonstrated by radioactive ISH (Figure 3A) and qPCR analysis (Supplemental Figure 1A) using thyroid glands from 2.5-month-old animals. Neither in Mct8 ko nor in Mct10 ko mice could a compensatory up-regulation of each other's expression be noted. Inactivation of Mct10 or Mct8 alone also did not affect transcript levels of deiodinases D1 and D2 as well as of genes involved in TH production, whereas the combined Mct10/Mct8 deficiency resulted in a significant increase in sodium/iodide symporter, thyroid peroxidase, and TSH receptor mRNA expression, suggesting that TH production may be stimulated in the absence of both transporters (Figure 3B).

In order to address this question we determined thyroidal TH concentrations in 2.5-month-old animals by measuring non-thyroglobulin-bound (= free) TH concentrations as well as total TH that reflect both free as well as thyroglobulin-bound TH. As depicted in Figure 3C, Mct10 ko mice did not show any alterations in thyroidal TH content whereas Mct8 ko animals exhibited significantly elevated free and total TH levels in agreement with previous observations (16, 18). In thyroid glands of Mct10/Mct8 dko animals, free and total T_4 content were 1.8-fold and 1.9-fold elevated compared with levels in Mct8 ko mice. Free and total T_3 content were found to be even further elevated because Mct10/Mct8 dko mice exhibited a 2.7-fold increase in free T_3 and a 2.5-fold rise in total T_3 compared with the values measured in Mct8 ko animals. Obviously, Mct10/Mct8 dko mice show a much higher accumulation of TH in the thyroid gland than Mct8 ko animals.

We wondered whether the strong up-regulation in thyroidal TH content of Mct10/Mct8 dko animals was reflected by an increase in follicle size. We therefore quantified the size of all follicles using 3–4 sections of each thyroid gland derived from animals of different age and genotype (Figure 4A). In agreement with our previous observations (16), we found a moderate increase (1.5- to

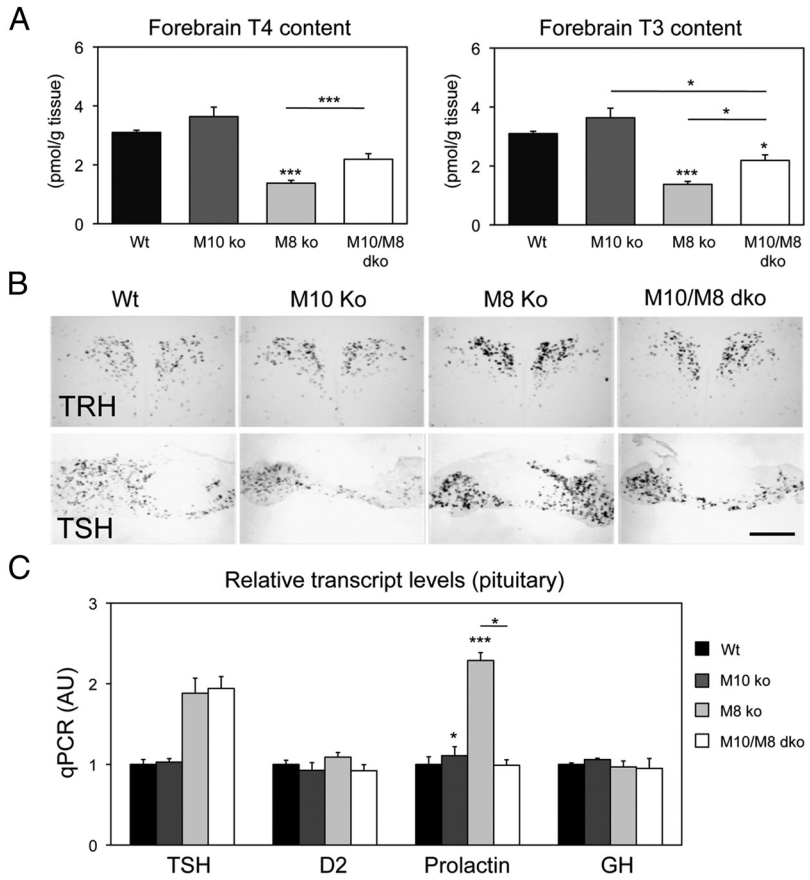

Figure 2. Consequences of Mct10 and Mct10/Mct8 deficiency on the TH status in brain and pituitary A, TH tissue content was measured in forebrain extracts of 2.5-month-old animals (n = 5 per genotype) and revealed normal concentrations in Mct10 (M10) ko mice. In contrast, T_4 and T_3 concentrations were significantly decreased in forebrains of Mct8 (M8) ko mice but to a lesser extent in Mct10/Mct8 (M10/M8) dko animals. B, Nonradioactive ISH studies were performed on brain and pituitary cryosections from P21-old animals (n = 3 per genotype) in order to visualize hypothalamic TRH (upper row) and pituitary TSH transcript levels (lower row), respectively. Wt and Mct10 ko animals showed a similar hybridization pattern, indicating no alterations in the respective transcript levels. As expected, TRH and TSH ISH intensities were visibly elevated in Mct8 ko mice whereas Mct10/Mct8 dko mice showed only elevated TSH but rather normal hypothalamic TRH signal intensities. Scale bar, 200 μm for TRH and 1 mm for TSH. C, qPCR analysis using pituitaries derived from 2.5-month-old animals (n = 5 per genotype) confirmed the observations from the ISH studies and revealed a similar rise in TSH transcript levels in Mct8 ko and Mct10/Mct8 dko mice. Type 2 deiodinase (D2) and GH transcript levels were not altered by Mct8 and/or Mct10 deficiency. Interestingly, prolactin expression showed a 2.2-fold increase in the pituitaries of Mct8 ko mice compared with Wt littermates. *, $P < .05$; **, $P < .005$; ***, $P < .001$. AU, arbitrary units.

2-fold) in follicle area in Mct8 ko mice. Mct10/Mct8-deficient animals tended to have even larger follicles than Mct8 ko mice although statistical significance could only be reached at 12 months of age. This difference between Mct8 ko and Mct10/Mct8 dko animals could not be explained by an enhanced TSH stimulation of the thyroid gland because Mct8 and Mct10/Mct8 dko animals exhibited similarly elevated serum TSH levels compared with Wt and Mct10 ko mice (Figure 4B).

By examining hematoxylin/eosin-stained thyroid gland sections, we observed another abnormality that was already described for Mct8 ko mice (33). At the age of 12 months, 63% of Mct8 ko mice and 20% of Mct10/Mct8 dko mice exhibited typical features of papillary microcarcinomas as evidenced by papillary-like outgrowth and enlarged as well as milk glass-like nuclei (Figure 4C). Moreover, a high number of proliferating thyroid follicle cells could be detected in these areas by Ki-67 immunostaining. We were not able to find either any histologic signs of microcarcinomas on sections derived from younger animals or from 12-month-old Wt or Mct10 ko animals.

Because the expression of tumor marker proteins may be already elevated at an early age, we performed qPCR analysis with thyroid glands of 2.5-month-old animals in order to determine the transcript levels of the pituitary tumor-transforming gene (Pttg1), Pttg1-binding factor (PBF) as well as of galectin-3 (Gal3). Pttg1 and PBF have been described as potent protooncogenes correlating with thyroid carcinomas (34, 35) whereas Gal3 is a well-established biomarker for thyroid carcinomas (36). As depicted in Figure 4D, neither Pttg1 nor PBF transcript levels were found to be elevated in Mct8- and/or Mct10-deficient animals. However, Gal3 mRNA levels were significantly higher in 2.5-month-old Mct8 ko mice and even more so in Mct10/Mct8-deficient animals, indicating an early onset of thyroidal derangements.

Renal TH transport and metabolism in Mct10- and Mct8-deficient animals

Mct10 and Mct8 are highly expressed in renal proximal tubule cells in which both transporters are localized at the basolateral membrane (17, 19). Inactivation of either Mct8 or Mct10 did not affect each other's transcript levels as illustrated by ISH and qPCR analysis (Supplemental Figure 1B). Determination of renal T_4 and T_3 content in 2.5-month-old animals revealed a 1.8-fold and a 5.3-fold increase in Mct8 ko mice compared with Wt littermates, whereas the tissue TH content in kidneys of Mct10 ko

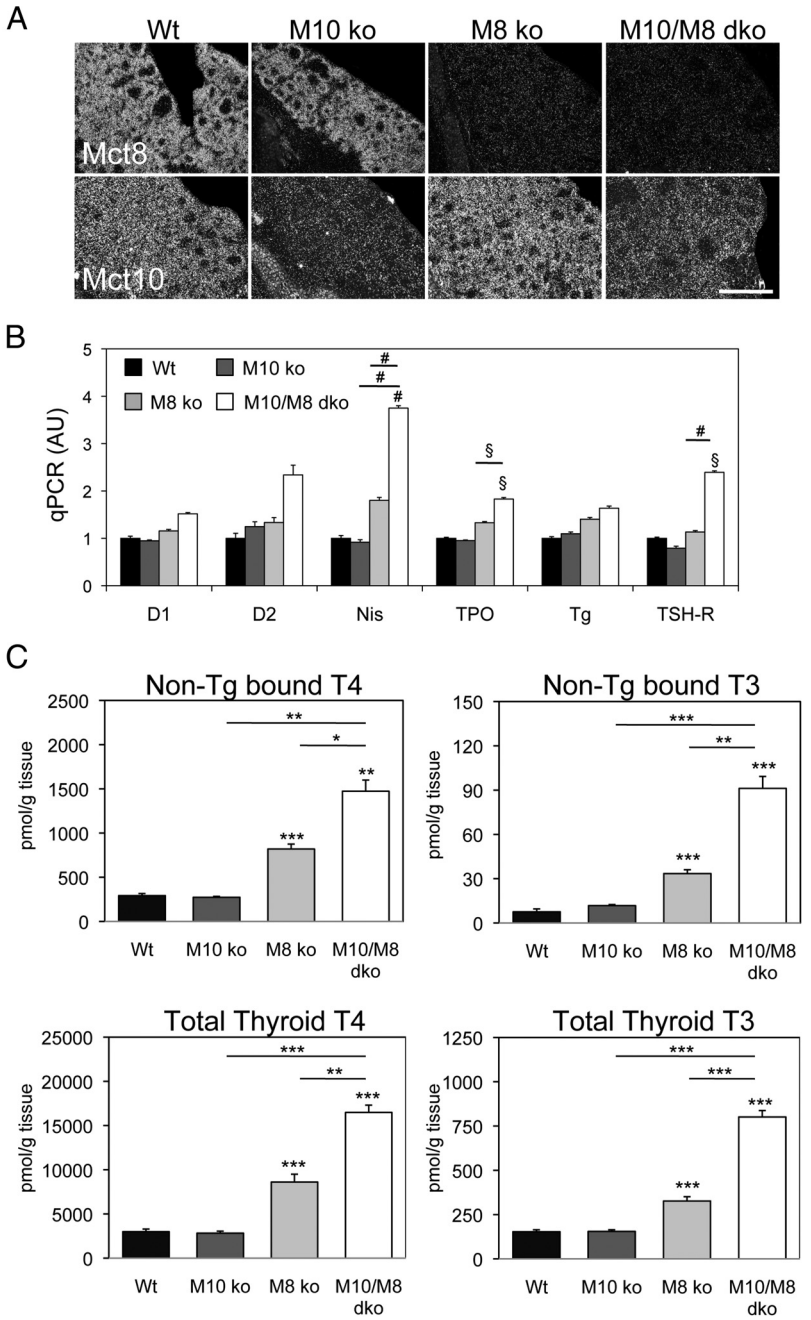

Figure 3. Consequences of Mct10 and/or Mct8 deficiency on the activity of the thyroid gland A, Radioactive ISH studies were performed on thyroid sections from 2.5-month-old animals (n = 3 per genotype) and revealed an overlapping expression of Mct8 and Mct10 in follicular epithelial cells in Wt animals. Inactivation of Mct8 or Mct10 did not visibly affect the transcript levels of the other TH transporter. Scale bar, 50 μm. B, Thyroidal expression profiling by qPCR showed significantly elevated transcript levels of the sodium/iodide symporter (Nis), thyroid peroxidase (TPO), and TSH receptor (TSH-R) in Mct10/Mct8 dko mice compared with Wt animal whereas neither Mct8 ko nor Mct10 ko animals revealed any significant alterations (n = 7 per genotype; age: 2.5 months). §, $P < .005$; #, $P < .001$. C, Non-thyroglobulin (Tg)-bound (= free) TH and total tissue TH concentrations were measured in thyroid glands of 2.5-month-old animals (n = 5 per genotype). Whereas Mct10 ko mice did not show any alterations, Mct8 ko mice displayed significantly elevated non-Tg-bound and total TH concentrations in the thyroid gland. Interestingly, these values were even further elevated in Mct10/Mct8 dko mice with the highest rise in non-Tg-bound T_3 (12-fold higher than Wt levels). *, $P < .05$; **, $P < .005$; ***, $P < .001$. AU, arbitrary units.

mice was not altered (Figure 5A). Intriguingly, significantly higher TH concentrations were observed in kidneys of Mct10/Mct8 dko mice with a 2.6-fold elevation in T_4 values and a 9.4-fold increase in T_3 levels compared with Wt mice. Obviously, the additional inactivation of Mct10 further enhanced the accumulation of TH in the kidneys of Mct8 ko mice.

In line with the increased renal T_3 values, determination of kidney D1 expression by ISH and qPCR revealed highly elevated D1 transcript levels in Mct8 ko animals whereas no changes in renal D1 expression could be observed in Mct10 ko mice (Figure 5, B and C). Again, Mct10/Mct8 dko animals showed the most pronounced alterations with a 3.8-fold rise in D1 compared with the 2.5-fold elevation in D1 mRNA levels found in Mct8 ko mice.

Because we recently found an increased renal accumulation of peripherally injected T_4 in Mct8 ko mice (17), we wondered whether Mct10/Mct8 dko animals would show an even higher renal uptake of T_4 than Mct8 ko mice. We therefore performed in vivo transport studies by injecting adult animals ip with ^{125}I-labeled T_4. As illustrated in Figure 5D, Mct10 ko mice showed similar amounts of renal radioactivity as Wt animals. In comparison, renal radioactivity was significantly elevated in both Mct8 ko and Mct10/Mct8 dko mice at all analyzed time points, suggesting that renal T_4 uptake was similarly stimulated in Mct8 ko and Mct10/Mct8 dko mice.

Analysis of TH metabolism and transport in the liver of Mct10- and Mct8-deficient mice

In addition to the kidneys, Mct10 and Mct8 show also an overlapping expression in the liver as demonstrated by ISH (Supplemental Figure 1C). Whereas Mct10 deficiency did

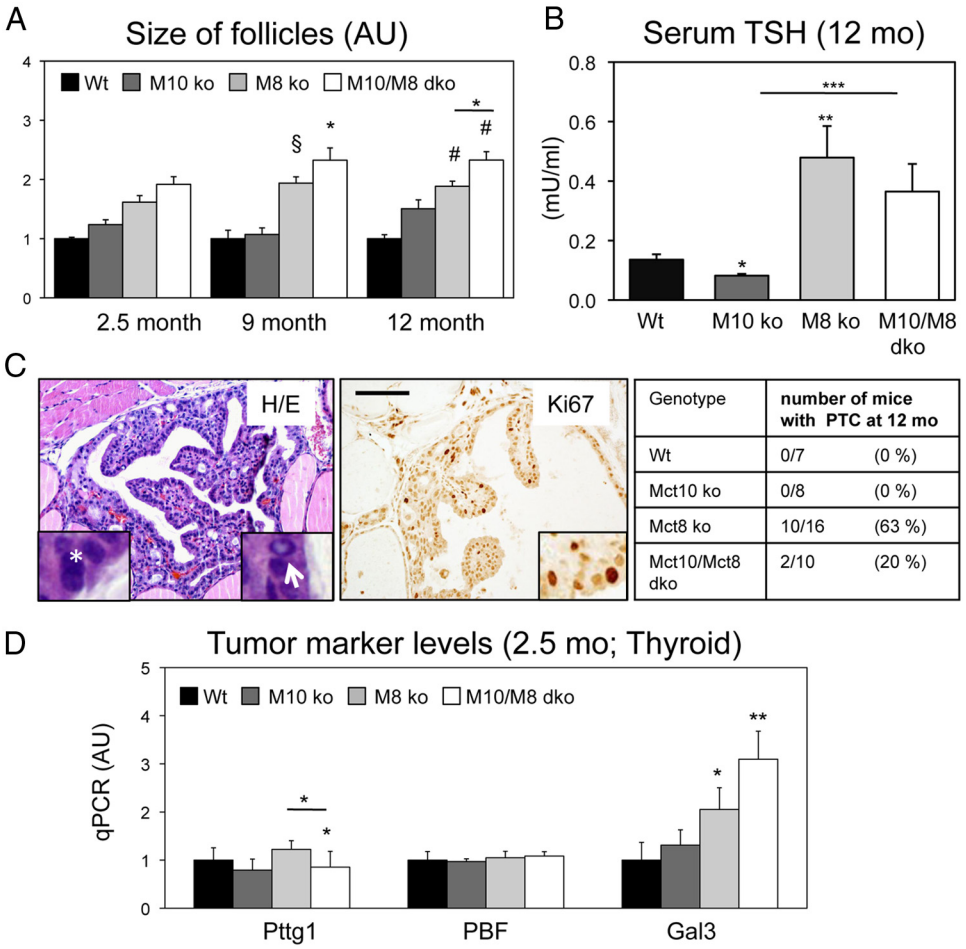

Figure 4. Follicular abnormalities in Mct8-deficient thyroid glands A, Follicle areas were quantified with ImageJ using hematoxylin/eosin (H/E)-stained thyroid paraffin sections derived from adult animals (at least 3 animals per genotype and time point). A significant increase in follicle size was found only in Mct8 ko and Mct10/Mct8 dko animals. B, Serum TSH levels were determined in 12-month-old animals (n = 6 per genotype) and revealed similarly elevated concentrations in Mct8 ko and Mct10/Mct8 dko mice. C, Papillary microcarcinoma-H/E stained thyroid sections from 12-month-old Mct8 ko animals that showed papillary-like outgrowth, enlarged nuclei (asterisk), and milk glass-like nuclei (arrow). Enlarged follicles are visible around this lesion. Immunostaining of thyroid sections with the proliferation marker Ki-67 revealed a high number of mitotic cells within the papillary-like structures. Scale bar, 50 μm. D, Thyroidal expression of the different tumor-associated markers was determined in 2.5-month-old animals (n = 7 per genotype) by qPCR and revealed significantly elevated Galectin-3 (Gal3) in Mct8 ko and Mct10/Mct8 dko mice. Pttg1, pituitary tumor-transforming gene 1; *, $P < .05$; **, $P < .005$; ***, $P < .001$. AU, arbitrary units.

not affect hepatic Mct8 transcript levels, ISH and qPCR revealed a slight increase in hepatic Mct10 mRNA levels in Mct8 ko animals. Neither liver TH content nor D1 mRNA levels were altered in Mct10 ko mice (Figure 6, A and B). In comparison, Mct8 ko mice displayed normal hepatic T_4 levels but a 3.7-fold rise in liver T_3 levels. Strikingly, Mct10/Mct8 dko mice showed a 1.6-fold elevation in liver T_4 and a 5-fold rise in hepatic T_3 levels. In agreement with the elevated liver T_3 values, ISH and qPCR analysis revealed increased hepatic transcript levels for the T_3-regulated genes D1 and GPD2 in Mct8 ko mice and even higher levels in Mct10/Mct8 dko mice (Figure 5B and Supplemental Figure 1, D and E). In vivo transport studies, however, did not exhibit any alterations in hepatic [^{125}I]T_4 uptake between Wt, Mct10 ko, and Mct8 ko mice. Only in liver samples of Mct10/Mct8 dko animals did we detect a significant increase in radioactivity 1 and 3 hours after [^{125}I]T_4 ip injections (Figure 6C).

Finally, we analyzed established serum markers of TH action and found significantly increased concentrations of alkaline phosphatase as well as significantly reduced amounts of cholesterol in the serum of Mct8 ko and Mct10/Mct8 dko animals (Figure 6D). These changes in serum parameters are indicative of a pronounced hepatic thyrotoxicosis and have already been reported for Mct8 ko mice (7, 37).

Discussion

Both in humans and mice, Mct8 deficiency is associated with robust alterations in the TH serum profile charac-

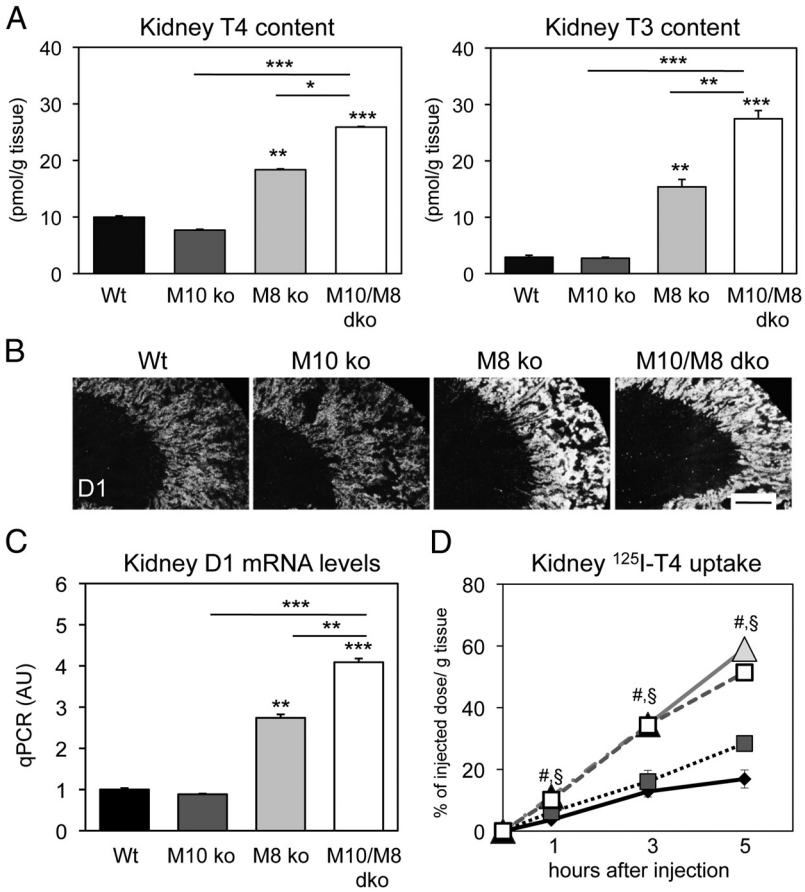

Figure 5. Excessive TH levels in the kidneys of Mct8 ko and Mct10/Mct8 dko mice A, The tissue concentrations of T_4 and T_3 were determined in kidneys of 2.5-month-old animals (n = 5 per genotype) and revealed highest levels in the absence of both TH transporters (Mct10/Mct8 dko). B, Radioactive ISH analysis demonstrated elevated type 1 deiodinase (D1) transcript levels in the renal cortex of Mct8 ko and Mct10/Mct8 dko mice and highest D1-specific signal intensities in the renal outer medulla of Mct10/Mct8-deficient animals. Scale bar, 1 mm. C, In agreement with the ISH data, qPCR analysis showed strongly elevated D1 transcript levels in Mct8 ko mice and a further increase in Mct10/Mct8 dko animals (n = 7 per genotype, P21). **, $P < .005$; ***, $P < .001$. D, Quantification of renal radioactivity following the ip injection of [^{125}I]T_4 revealed a similar increase over time in Mct8 ko (gray triangle) and Mct10/Mct8 dko (open square) animals whereas the uptake of [^{125}I]T_4 in Mct10 ko (gray square) mice was similar to that of Wt (black diamond) animals. #, $P < .05$ for Mct8 ko vs Wt; §, $P < .001$ for Mct10/Mct8 dko vs Wt.

terized by high serum T_3 in the presence of rather low circulating T_4 serum levels. Although the exact underlying pathogenic mechanisms are still not fully understood, studies of Mct8 ko mouse mutants had shed some light on this issue. Obviously, a disturbed TH homeostasis in several tissues contributes to the generation of the imbalanced T_3:T_4 ratio. In the absence of Mct8, the thyroid gland itself is affected because, despite an increase in thyroidal TH content, less T_4 is secreted into the bloodstream upon TSH stimulation. This observation indicates that Mct8 fulfils an important T_4 export function in the thyroid (16, 18). In the liver and kidneys, high D1 activities lead to an increased T_4 to T_3 conversion that in turn seems to contribute significantly to the elevated serum T_3 levels (38). Finally and most surprisingly, absence of Mct8 is associated with an elevated transport of T_4 and T_3 into the kidneys that, in turn, leads to a thyrotoxic state. Based on these findings, it is tempting to speculate that in peripheral organs such as liver, thyroid, and kidneys, Mct8 may preferentially act as a T_4 efflux system whereas the import/export of T_3 may still be ensured by other TH transporters. This scenario is different from the situation in the murine brain in which Mct8 deficiency is associated with an impaired transport of T_3 across the BBB whereas T_4 uptake was found to be only mildly compromised (8, 9). The latter observation can be explained by the presence of the T_4-selective transporter Oatp1c1, which can partially compensate for the absence of Mct8 in the mouse CNS. Indeed, the generation and analysis of Mct8/Oatp1c1 dko mice underscored the physiologic significance of this TH transporter pair as the passage of TH into the brain was highly diminished (15). However, because Oatp1c1 expression is restricted to the CNS, it remains to be elucidated which transporters may functionally cooperate with Mct8 in mediating TH transport in peripheral tissues.

Here, we followed the hypothesis that Mct10, the T-type amino acid transporter TAT1, functions as a transporter for TH as well. In vitro transport studies using transfected COS1 cells already revealed that human MCT10 transports T_3 at least as efficiently as human MCT8 in and out of cells whereas it is less active toward the transport of T_4 (22). In mice, Mct10 shows an overlapping expression with Mct8 in organs such as liver, kidney, and thyroid in which Mct10 may act together with Mct8 in mediating cellular TH passage.

Unexpectedly, our analysis of Mct10-deficient animals did not reveal any abnormalities in serum TH levels or TH homeostasis, questioning the physiologic significance of Mct10 as a prominent TH transporter in vivo. However, this conclusion may be misleading and the "real" contribution of Mct10 in TH transport may be masked due to the presence of Mct8, which might be able to compensate for the absence of Mct10 in Mct10 ko mice. We therefore

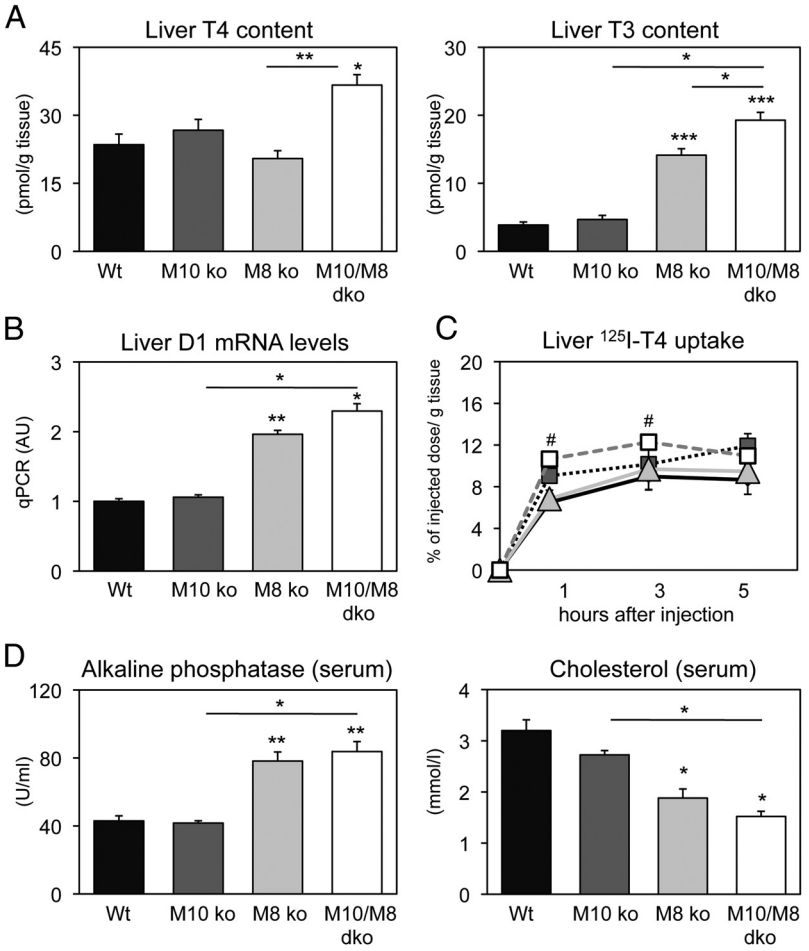

Figure 6. Accumulation of T_4 and T_3 in the liver of Mct10/Mct8 dko mice A, Determination of hepatic TH concentrations revealed highly elevated T_3 and T_4 levels of Mct10/Mct8 dko mice whereas Mct8 ko mice only exhibited an increase in hepatic T_3 (n = 5 per genotype; 2.5 months old). B, Both Mct8 ko and Mct10/Mct8 dko mice showed a rise in liver D1 transcript levels as assessed by qPCR analysis (n = 7 per genotype; P21). C, After the injection of ^{125}I-labeled T_4, animals (n = 3–5 per time point and genotype; 2–6 months old) were perfused with saline at given time points. Amounts of radioactivity in liver samples were quantified and presented as the percentage of injected dose normalized to the respective tissue weight. No differences in hepatic accumulation of radioactivity could be observed between the different genotypes. #, $P < .005$ for Mct10/Mct8 dko (open square) vs Wt (black diamond); light gray triangle, Mct8 ko; gray square, Mct10 ko. D, Quantification of serum parameters demonstrated increased alkaline phosphatase and decreased cholesterol concentrations in Mct8 ko and Mct10/Mct8 dko mice (n = 5 per genotype; 6–8 months old). These alterations are indications for a thyrotoxic state of the liver. *, $P < .05$; **, $P < .005$; ***, $P < .001$. AU, arbitrary units.

generated and analyzed mouse mutants that were deficient in both TH transporters and, indeed, we found robust alterations in these Mct10/Mct8 dko mice that by far exceeded those characteristic changes in tissue TH homeostasis caused by Mct8 deficiency alone. In particular, TH tissue concentrations were highly elevated in the liver and kidney of Mct10/Mct8 dko mice, indicating that these organs are even more "hyperthyroid" in the absence of both TH transporters than in the single Mct8 ko mice. Intriguingly, in vivo transport studies did not reveal any differences in renal and hepatic T_4 uptake between Mct8 ko and Mct10/Mct8 dko mice. It is therefore tempting to speculate that Mct8 and Mct10 are primarily involved in facilitating the efflux of TH whereas other unidirectional transport systems are critical for the import of TH, particularly into the liver and kidney.

In addition to liver and kidney, the thyroid gland appears to be more severely affected if both TH transporters are missing. Total TH as well as non-thyroglobulin-bound TH concentrations in the thyroid glands were approximately 2- to 3-fold higher in Mct10/Mct8 dko mice compared with the already elevated thyroidal TH levels of Mct8 ko mice. This remarkable rise may reflect, at least in part, a stimulated thyroidal TH production because transcript levels of sodium/iodide symporter, thyroid peroxidase, and TSH-receptor were found to be highly elevated specifically in Mct10/Mct8 dko mice whereas serum TSH levels were similarly increased in Mct8 ko and Mct10/Mct8 dko mice. On the other hand, the pronounced accumulation of particularly free T_3 in the thyroid glands of Mct10/Mct8 dko mice suggests that, in addition to a compromised T_4 secretion due to the absence of Mct8, the additional lack of Mct10 is associated with a further restriction in T_3 efflux.

Previous histologic studies have revealed that starting from the age of 6 months, Mct8 ko mice gradually developed papillary hyperplasia and nuclear features consistent with papillary thyroid carcinoma (PTC) (33). Wirth et al (33) also reported pronounced thyroidal abnormalities including hyperplastic nodules and microfollicular structures in one patient with MCT8 mutation. Here, we could detect similar morphologic alterations in 12-month-old animals with a higher incidence in Mct8 ko mice compared with Mct10/Mct8 dko mice. Moreover, we could detect highly increased galectin-3 transcript levels in thyroid glands of Mct8 ko and Mct10/Mct8 dko animals already at 2.5 months of age, indicating that the onset of the papillary changes occurs at a rather young age but progresses very slowly. Whether the chronically increased serum TSH levels drives the development of the

PTC or additional cell-intrinsic factors promote follicular abnormalities during Mct8 deficiency is currently a matter of speculation, and more sophisticated animal models are required to answer this question.

The most puzzling observation was the finding that the additional inactivation of Mct10 in Mct8 ko mice leads to a normalization of the low serum T_4 concentrations without normalizing the high serum T_3 levels. The normal serum T_4 appears to have beneficial effects on the CNS because brain TH content was less severely decreased and, consequently, hypothalamic TRH expression less affected in Mct10/Mct8 dko mice compared with Mct8 ko animals. Because Mct10 is absent from the murine BBB and Mct8 plays only a minor role in facilitating T_4 uptake into the mouse CNS (8), it may not be too surprising that due to the rise in serum T_4 in Mct10/Mct8 dko mice, brain T_4 content is higher in these animals compared with Mct8 ko mice that show low serum T_4 and consequently decreased T_4 brain content.

Why the drop in serum T_4 in Mct8-deficient animals can be prevented by the additional inactivation of Mct10 remains to be investigated. We and others have speculated that the low serum T_4 levels in Mct8 ko mice are due to a decreased thyroidal T_4 secretion as well as an increased T_4 to T_3 conversion mediated by D1 in liver and kidneys (16–18). In support of this hypothesis, Mct8/D1 dko animals showed rather normal serum TH levels, suggesting that the rise in hepatic and renal D1 expression in Mct8 ko mice contributes significantly to the odd T_3/T_4 serum profile (38). We also proposed that, in the absence of Mct8, the kidney acts as a "sink" for T_4 because Mct8 ko mice exhibited strongly increased renal T_4 concentrations despite the low serum T_4 (17). However, Mct10/Mct8 dko mice displayed highly elevated hepatic and renal D1 expression together with a further accumulation of TH in liver and kidney. These findings indicate that the normalization of serum T_4 levels in Mct10/Mct8 dko mice is not a consequence of a decreased hepatic and renal T_4 uptake and metabolism and that further studies are needed to fully understand the tissue-specific role of these 2 transporters.

In summary, our data could support the notion that Mct10 indeed acts as a TH transporter in vivo although its contribution only becomes evident when Mct8 as the most prominent TH transporter is missing. In light of the seemingly normal TH homeostasis in Mct10 ko mice, it is not too surprising that patients carrying mutations in the *MCT10* gene have not been identified so far and that single nucleotide polymorphisms in the *MCT10* gene could not be linked to alterations in serum TH levels (39, 40). Remarkably, inactivation of Mct10 in Mct8 ko mice resulted in normal serum T_4 levels and, consequently, ameliorated the brain thyroidal state. At first glance, such an outcome might be beneficial for patients with MCT8 mutations because an increase in serum T_4 may ultimately improve the psychomotor development. However, normalization of the serum T_4 concentration is found only in the adult Mct10/Mct8 dko animals and thus occurs too late to affect neural differentiation processes. Moreover, our data clearly indicate that, in the absence of both transporters, the thyrotoxic situation in liver, kidney, and thyroid gland becomes even more pronounced than in the single Mct8 ko mice. Consequently, the development of Mct10-specific inhibitors does not appear to represent a possible therapeutic strategy to manipulate serum TH levels in patients with *MCT8* mutations. Rather, further studies using conditional Mct8 and Mct10 mouse mutants are needed to shed light on the tissue-specific function of these TH transporters as well as on the exact pathogenic mechanisms that underlie the development of the abnormal serum TH profile.

Acknowledgments

We thank Sabine Landmann (Fritz Lipmann Institute, Jena), H.C. van Beeren (Academic Medical Center), Ramona van Heerebeek (Erasmus Medical Center, Rotterdam), Lut Noterdaeme, and Lieve Geenen (Katholieke Universiteit Leuven) for excellent technical assistance.

Address all correspondence and requests for reprints to: Heike Heuer, PhD, Leibniz Institute for Age Research/Fritz Lipmann Institute, Beutenbergstrasse 11, D-07745 Jena, Germany. E-mail: hheuer@fli-leibniz.de.

This work was supported by Deutsche Forschungsgemeinschaft Grants HE3418/5–1 and SPP1629 (HE3418/7–1).

Disclosure Summary: The authors have nothing to disclose.

References

1. **Visser WE, Friesema EC, Jansen J, Visser TJ.** Thyroid hormone transport in and out of cells. *Trends Endocrinol Metab*. 2008;19:50–56.
2. **Friesema EC, Ganguly S, Abdalla A, et al.** Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. *J Biol Chem*. 2003;278:40128–40135.
3. **Dumitrescu AM, Liao XH, Best TB, Brockmann K, Refetoff S.** A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. *Am J Hum Genet*. 2004;74:168–175.
4. **Friesema EC, Grueters A, Biebermann H, et al.** Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet*. 2004;364:1435–1437.
5. **Schwartz CE, May MM, Carpenter NJ, et al.** Allan-Herndon-Dudley syndrome and the monocarboxylate transporter 8 (MCT8) gene. *Am J Hum Genet*. 2005;77:41–53.
6. **Friesema EC, Jansen J, Heuer H, Trajkovic M, Bauer K, Visser TJ.** Mechanisms of disease: psychomotor retardation and high T_3 levels

caused by mutations in monocarboxylate transporter 8. *Nat Clin Pract Endocrinol Metab*. 2006;2:512–523.
7. **Dumitrescu AM, Liao XH, Weiss RE, Millen K, Refetoff S.** Tissue-specific thyroid hormone deprivation and excess in monocarboxylate transporter (mct) 8-deficient mice. *Endocrinology*. 2006;147:4036–4043.
8. **Trajkovic M, Visser TJ, Mittag J, et al.** Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. *J Clin Invest*. 2007;117:627–635.
9. **Ceballos A, Belinchon MM, Sanchez-Mendoza E, et al.** Importance of monocarboxylate transporter 8 for the blood-brain barrier-dependent availability of 3,5,3′-triiodo-L-thyronine. *Endocrinology*. 2009;150:2491–2496.
10. **Wirth EK, Roth S, Blechschmidt C, et al.** Neuronal 3′,3,5-triiodothyronine (T_3) uptake and behavioral phenotype of mice deficient in Mct8, the neuronal T_3 transporter mutated in Allan-Herndon-Dudley syndrome. *J Neurosci*. 2009;29:9439–9449.
11. **Sugiyama D, Kusuhara H, Taniguchi H, et al.** Functional characterization of rat brain-specific organic anion transporter (Oatp14) at the blood-brain barrier: high affinity transporter for thyroxine. *J Biol Chem*. 2003;278:43489–43495.
12. **Tohyama K, Kusuhara H, Sugiyama Y.** Involvement of multispecific organic anion transporter, Oatp14 (Slc21a14), in the transport of thyroxine across the blood-brain barrier. *Endocrinology*. 2004;145:4384–4391.
13. **Ito K, Uchida Y, Ohtsuki S, et al.** Quantitative membrane protein expression at the blood-brain barrier of adult and younger cynomolgus monkeys. *J Pharm Sci*. 2011;100:3939–3950.
14. **Roberts LM, Woodford K, Zhou M, et al.** Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood-brain barrier. *Endocrinology*. 2008;149:6251–6261.
15. **Mayerl S, Visser TJ, Bauer R, Darras VM, Heuer H.** Consequences of brain-specific thyroid hormone deprivation im Mct8/Oatp1c1 double knockout mice. *Thyroid*. 2012;22:A-2 (abstract). 82nd Annual Meeting of the American Thyroid Association, Québec City, Canada.
16. **Trajkovic-Arsic M, Müller J, Darras VM, et al.** Impact of monocarboxylate transporter-8 deficiency on the hypothalamus-pituitary-thyroid axis in mice. *Endocrinology*. 2010;151:5053–5062.
17. **Trajkovic-Arsic M, Visser TJ, Darras VM, et al.** Consequences of monocarboxylate transporter 8 deficiency for renal transport and metabolism of thyroid hormones in mice. *Endocrinology*. 2010;151:802–809.
18. **Di Cosmo C, Liao XH, Dumitrescu AM, Philp NJ, Weiss RE, Refetoff S.** Mice deficient in MCT8 reveal a mechanism regulating thyroid hormone secretion. *J Clin Invest*. 2010;120:3377–3388.
19. **Ramadan T, Camargo SM, Summa V, et al.** Basolateral aromatic amino acid transporter TAT1 (Slc16a10) functions as an efflux pathway. *J Cell Physiol*. 2006;206:771–779.
20. **Kim DK, Kanai Y, Chairoungdua A, Matsuo H, Cha SH, Endou H.** Expression cloning of a Na+-independent aromatic amino acid transporter with structural similarity to H+/monocarboxylate transporters. *J Biol Chem*. 2001;276:17221–17228.
21. **Kim DK, Kanai Y, Matsuo H, et al.** The human T-type amino acid transporter-1: characterization, gene organization, and chromosomal location. *Genomics*. 2002;79:95–103.
22. **Friesema EC, Jansen J, Jachtenberg JW, Visser WE, Kester MH, Visser TJ.** Effective cellular uptake and efflux of thyroid hormone by human monocarboxylate transporter 10. *Mol Endocrinol*. 2008;22:1357–1369.
23. **Ramadan T, Camargo SM, Herzog B, Bordin M, Pos KM, Verrey F.** Recycling of aromatic amino acids via TAT1 allows efflux of neutral amino acids via LAT2–4F2hc exchanger. *Pflugers Arch*. 2007;454:507–516.
24. **Mariotta L, Ramadan T, Singer D, et al.** T-type amino acid transporter TAT1 (Slc16a10) is essential for extracellular aromatic amino acid homeostasis control. *J Physiol*. 2012;590:6413–6424.
25. **Friedrichsen S, Christ S, Heuer H, et al.** Regulation of iodothyronine deiodinases in the Pax8−/− mouse model of congenital hypothyroidism. *Endocrinology*. 2003;144:777–784.
26. **Wiersinga WM, Chopra IJ.** Radioimmunoassay of thyroxine (T_4), 3,5,3′-triiodothyronine (T_3), 3,3′,5′-triiodothyronine (reverse T_3, rT_3), and 3,3′-diiodothyronine (T_2). *Methods Enzymol*. 1982;84:272–303.
27. **Reyns GE, Janssens KA, Buyse J, Kühn ER, Darras VM.** Changes in thyroid hormone levels in chicken liver during fasting and refeeding. *Comp Biochem Physiol B Biochem Mol Biol*. 2002;132:239–245.
28. **Pohlenz J, Maqueem A, Cua K, Weiss RE, Van Sande J, Refetoff S.** Improved radioimmunoassay for measurement of mouse thyrotropin in serum: strain differences in thyrotropin concentration and thyrotroph sensitivity to thyroid hormone. *Thyroid*. 1999;9:1265–1271.
29. **van Zeijl CJ, Surovtseva OV, Wiersinga WM, Boelen A, Fliers E.** Transient hypothyroxinemia in juvenile glycoprotein hormone subunit B5 knock-out mice. *Mol Cell Endocrinol*. 2010;321:231–238.
30. **Heuer H, Schäfer MK, O'Donnell D, Walker P, Bauer K.** Expression of thyrotropin-releasing hormone receptor 2 (TRH-R2) in the central nervous system of rats. *J Comp Neurol*. 2000;428:319–336.
31. **Tashjian AH Jr, Barowsky NJ, Jensen DK.** Thyrotropin releasing hormone: direct evidence for stimulation of prolactin production by pituitary cells in culture. *Biochem Biophys Res Commun*. 1971;43:516–523.
32. **Jacobs LS, Snyder PJ, Wilber JF, Utiger RD, Daughaday WH.** Increased serum prolactin after administration of synthetic thyrotropin releasing hormone (TRH) in man. *J Clin Endocrinol Metab*. 1971;33:996–998.
33. **Wirth EK, Sheu SY, Chiu-Ugalde J, et al.** Monocarboxylate transporter 8 deficiency: altered thyroid morphology and persistent high triiodothyronine/thyroxine ratio after thyroidectomy. *Eur J Endocrinol*. 2011;165:555–561.
34. **Kim CS, Ying H, Willingham MC, Cheng SY.** The pituitary tumor-transforming gene promotes angiogenesis in a mouse model of follicular thyroid cancer. *Carcinogenesis*. 2007;28:932–939.
35. **Smith VE, Read ML, Turnell AS, et al.** PTTG-binding factor (PBF) is a novel regulator of the thyroid hormone transporter MCT8. *Endocrinology*. 2012;153:3526–3536.
36. **Htwe TT, Karim N, Wong J, Jahanfar S, Mansur MA.** Differential expression of galectin-3 in advancing thyroid cancer cells: a clue toward understanding tumour progression and metastasis. *Singapore Med J*. 2010;51:856–859.
37. **Weiss RE, Murata Y, Cua K, Hayashi Y, Seo H, Refetoff S.** Thyroid hormone action on liver, heart, and energy expenditure in thyroid hormone receptor β-deficient mice. *Endocrinology*. 1998;139:4945–4952.
38. **Liao XH, Di Cosmo C, Dumitrescu AM, et al.** Distinct roles of deiodinases on the phenotype of Mct8 defect: a comparison of eight different mouse genotypes. *Endocrinology*. 2011;152:1180–1191.
39. **van der Deure WM, Peeters RP, Visser TJ.** Molecular aspects of thyroid hormone transporters, including MCT8, MCT10, and OATPs, and the effects of genetic variation in these transporters. *J Mol Endocrinol*. 2009;44:1–11.
40. **Medici M, van der Deure WM, Verbiest M, et al.** A large-scale association analysis of 68 thyroid hormone pathway genes with serum TSH and FT4 levels. *Eur J Endocrinol*. 2011;164:781–788.